SEPARATION OF A HIGH-VALUE MAIZE PROTEIN IN FUEL ETHANOL PRODUCTION

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ABSTRACT: Extraction of zein from maize (Zea mays) using ethanol solutions, followed by reduction of the extract's ethanol content, will precipitate a solid mixture of zein and maize lipid. For many zein applications, such as film production, purer zein is required than can be precipitated this way. Although most of the lipid can be removed from the precipitate by selective re-extraction with hexane or ethanol, it is less expensive to produce a low lipid content precipitate by diluting the extract in stages. An initial dilution will precipitate a high lipid content solid, which can be separated from the extract and followed by a further reduction of extract ethanol concentration, precipitation and centrifugation of a high zein content precipitate. The mass and composition of solid precipitates centrifuged from a maize extract that was diluted in several steps were measured. The lipid content of a zein product precipitated from an extract diluted from 55% ethanol to 50% is significantly lower than the content of precipitate of an extract diluted in a single step to 40% ethanol. The solubility of zein is bounded by ethanol: water mole ratios 2:1 to 1:2, with 1:1 being near the maximum solubility. These ratios are consistent with hexagonal rings comprised of alternating water and ethanol molecules adjacent to the zein solute.

1. INTRODUCTION

The Agricultural Research Service of the U.S. Department of Agriculture is developing a method of economically extracting zein from corn (maize) that will subsequently be fermented to fuel ethanol. The potential value of the non-fermentable maize components has been widely recognized and the search for applications beyond livestock feed has been extensive. Zein, a principal corn protein, is commercially extracted from corn gluten meal (CGM), but refined zein extracted from corn gluten costs 10$/lb and only a few tons are produced each year. Sales of greater amounts are inhibited by its cost. The CGM is separated from steeped maize after germ and fiber removal, as part of a wet milling ethanol process.

We are endeavoring to develop a zein extraction process suitable for dry-grind ethanol plants or wet mills with lower process costs. Zein extraction and sales can significantly reduce the overall cost of producing ethanol and sale of extracted zein will enable U.S. corn producers to obtain and maintain higher return from the non-starch components of corn. To encourage investment in the corn extraction process, its market must be defined. An initial market for the corn-extracted zein is replacing corn gluten-zein and other higher cost materials, as an edible coating or sheet-forming material. This article reports a method for improving the zein purity so that it will have acceptable coating properties.

The potential value of the non-fermentable maize components, beyond livestock feed, has been widely recognized and the search for new applications has been described (7, 4). Retrofitting existing dry mills will be the least expensive way to produce amounts of zein that will be needed to supply an initial market. Commercial uses for zein outside the food industry are not competitive and may take several years to develop.

In principle, zein could be separated from the solid product, distillers' dried grains (DDG), of existing dry grind or beverage alcohol plants. However, relatively little protein in DDG can be extracted without adding reducing compounds to the ethanol (9, 11, 12). For DDG, heating in the distillation reboiler, or dryer, reduces extractability of zein from these animal feed products (9) and CGM (10). Dry grind plant owners are loathe to separate solids from the finished fermentation process, ahead of the beer still, because of investment cost. Modulated differential scanning calorimetric measurements have shown differences in thermal properties between hot air-dried (commercial) CGM and freeze-dried CGM, attributed to browning reactions (2). Comparisons of CGM and endosperm analyses show that (6), CGM contains a significant amount of alcohol-extractable protein, which would not have been extractable from the endosperm.

Separation of zein and oil from maize by extraction with ethanol has been reported. Hojilla-Evangelista (3) described a process to dry a 95% ethanol stream to fuel grade with dry maize. The ethanol stream extracted oil from the corn, and zein was subsequently extracted from the deoiled maize, with 45% ethanol-55% 0.1 M NaOH. The scheme for recovering oil and protein in consecutive extractions appears to produce good recoveries, but the cost of evaporating the ethanol solutions from the maize twice is roughly twice that of a single extraction. Dickey et al. (1) described a single extraction process with 70% ethanol, followed by dilution of the extract to 40% ethanol and centrifugation to separate a solid containing 70-80% protein. For uses such as forming a grease-resistant coated paper, zein combined with the natural maize oil may be acceptable. Films made from mixtures of commercial zein and fatty acids have higher clarity, elongation and toughness but lower modulus, tensile strength and water absorption than pure zein films (5, 8). However, high purity zein is desirable for uses requiring minimal color and aroma. Color- and aroma-imparting compounds can be extracted from the zein product with a hydrophobic, lipid-extracting, solvent. Alternatively, lesser amounts of the undesired compounds can be extracted initially, by using a more aqueous extracting solvent. Unfortunately, below 70-80% ethanol a lower ethanol

content solvent will extract zein more slowly than one with more ethanol, increasing extraction cost. Thus, the least expensive method of obtaining purer zein by alcohol extraction would be to extract with an ethanol concentration high enough to maximize the extraction rate, and subsequently remove a substantial fraction of the immiscible oil and undesirable associated compounds, by dilution, prior to dilution of the extract sufficient to precipitate zein.

In this study, maize was fine-cut by a commercial feed mill using a counter-rotating, ribbed disc mill. After extraction with 70% ethanol, solids were removed from the extract by successive dilutions followed by centrifugation. Diluted samples were re-concentrated by microfiltration and evaporation. The mass and composition of solids centrifuged from the maize extract were measured. One-third of the lipids extracted with 70 wt% ethanol were removed by centrifugation, after dilution to a concentration between 58 and 60 wt ethanol. After further dilution to 50% ethanol, 90% of the extracted zein was removed by centrifugation. Variation in ultracentrifuge speed showed that 10,000 rpm was significantly better at removing lipids from the 60% ethanol solution than separations at the lower speeds tried, but separation of zein from the 50% ethanol solution was insensitive to speed over the range 6-10,000 rpm.

This separation permits removal of a high-purity protein fraction from corn before fermentation, realizing higher quality, and reducing the volume of feed to the fermentor. The lipid fraction, to be studied separately, has value, and the only solvent is already internal to the process.

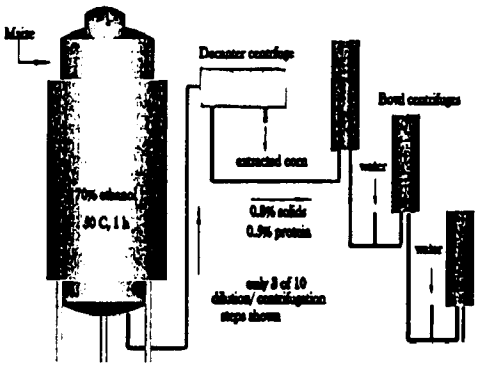
1.1 Continuous Centrifugation

The extraction and preliminary extract separation steps were carried out using 74 kg of extract, which was pumped through a bowl centrifuge at 17.7 kg/h. Solids, mostly fine particles not captured by the decanter centrifuge, weighing 151.7 gm. were recovered. The 72% ethanol centrifugate was diluted, held overnight, and pumped through the bowl centrifuge at 16 kg/h. This dilution, holding, and centrifugation sequence was repeated 10 times, with successively lower ethanol fraction. Based on the analysis of the precipitates, the extract contained 0.8% solids and 0.5% protein. No evidence of gelling was observed in the extracts or diluted extracts even after holding at ambient conditions for 2 months.

Table I
Extract dilution and continuous centrifugation

Ethanol (wt%)	Ppt. Mass (gm)	Solid (wt%)	Protein (wt%)	Lipid (wt%)	Time (days)[a]
63	6.4	98.1	14.9	74.3	2
58	28.4	98.8	12.7	86.5	3
53	57.0	92.9	49.3	31.2	7
50	347.3	88.6	75.5	7.1	8
47	55.9	95.0	59.5	11.6	9
43	14.2	97.4	42.6	38.7	10
39	17.1	96.6	36.0	30.2	13
33	17.8	98.3	48.2	5.4	14
27	4.0	97.4	45.9	2.6	15
19	3.9	95.2	39.5	15.3	16

[a]Time interval between extraction and centrifugation.



1.2 Batch centrifugation

The decanted liquid extract, 81.3 kg, was collected, held overnight and pumped through a bowl centrifuge at 16.3 kg/h. Fine particles not separated by the decanter centrifuge, 189.4 g, were removed from the extract and deposited on the bowl wall. The extract, 73.5 kg, was divided into two parts. The smaller part, 36.5 kg, was diluted with 6.5 kg of tap water and held overnight. The 37 kg of decanted extract remaining was stored for several months and used later for tests of concentrated extract. The diluted part of the extract was pumped through the bowl centrifuge and 40 kg of centrifugate recovered; a 5-kg portion of the centrifugate was used for a series of dilution/ holding / batch centrifugations similar to those carried out with the bowl centrifuge.

The batch centrifugations were carried out using an RC-5B Sorvall automatic superspeed refrigerated centrifuge (Kendro Laboratory Products, Newtown, CT) with GSA rotor. At 10,000 RPM the centrifuge produced 13,300 × g. The samples were spun for 30 min, while being held at 0 C.

Table II
Extract dilution and batch centrifugation

Ethanol (wt%)	Ppt. Mass (gm)	Solid (wt%)	Protein (wt%)	Lipid (wt%)	Time (days)[a]
60	10.7	98.8	10.6	87.2	2
57	43.8[b]	94.4	72.5	11.6	6
55	0	-	-	-	7
53	9.6	96.0	71.7	15.2	8
51	39.8	92.8	86.2	8.2	9
49	67.7	93.6	78.6	10.3	12
47	18.3	96.2	76.8	9.3	13
45	3.2	98.1	70.9	-	14
43	0.8	-	46.9		15
42	0				16
40	0				20
38	0.8	99.2	48.3	-	21
36	1.6	99.1	47.5	-	22
34	0.8	-	44.1	-	23
27	1.6	98.9	28.9	-	26

[a]Time interval between extraction and centrifugation.
[b] 5 kg of the 40 kg of 63% ethanol diluted extract was diluted to produce this diluted extract, this and subsequent solid masses was multiplied by 8 to account for this sampling.

Analysis of the precipitates from the batch centrifugation series indicated that the extract solution contained 0.6% solids and 0.4% protein. The 30-minute batch centrifugation is sufficiently long that the centrifugate would have an equilibrium concentration of solute, for the ethanol concentration. The solid composition and yield of each step of this series is shown in Table II.

2. CONCLUSIONS

A substantial fraction of the lipids extracted with the zein can be removed by centrifugation from 70% ethanol extracts of corn diluted to a concentration between 58 and 60% ethanol. This diluted solution contained 0.5 % zein and one-third of the lipid in the decanted extract was removed. After further dilution to 50% ethanol, 90% of the extracted zein was removed by continuous centrifugation. Variation in ultracentrifuge speed showed that 10,000 rpm is significantly better at removing lipids from the 60 % ethanol solution than separations at the lower speeds tried. However, separation of zein from the 50 % ethanol solution was insensitive to speeds over the range 6- 10,000 rpm.

3. REFERENCES

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